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c-erbB-2 Protein Overexpression and p53 Immunoreaction in Primary and Recurrent Breast Cancer Tissues

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Background and Objectives: We investigated whether expression levels of *c-erbB-2* and p53 proteins in breast cancer tissues differ in primary and metastatic lesions.
Methods: Immunohistochemical staining or sandwich enzyme immunoassay was used to determine expression levels of *c-erbB-2* and p53 proteins in 42 breast cancer samples from 21 patients. Estrogen (ER) and progesterone receptors (PgR) were also measured by enzyme immunoassay in each case. All patients had undergone radical surgery for primary tumors and surgical resection of asynchronous metastatic lesions. Thirteen patients (62%) were premenopausal and 14 (67%) received postoperative adjuvant therapies. Median disease-free survival time was 26 months (range, 5–104). The resected metastatic lesions included 1 in the liver, 3 in the lung, and 3 in the supraclavicular lymph nodes. The remaining 14 were local skin lesions.
Results: There was no difference in the positivity rate of *c-erbB-2* (38%: 8/21) and p53 (39%: 7/18) expression between the primary tumors and the recurrent lesions. In addition, no discordant *c-erbB-2* or p53 expression was observed between the primary tumors and their respective metastatic lesions. Positivity rates for ER and PgR were 50% (10/20) and 60% (12/20) for the primary tumors, but only 25% (5/20) and 30% (6/20) for the recurrent lesions, respectively ($P = 0.19$ for ER and $P = 0.11$ for PgR).
Conclusions: *c-erbB-2* and p53 expression levels in breast cancer cells were almost unchanged as the disease progressed and/or in response to adjuvant therapies, regardless of the hormone receptor status.
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KEY WORDS: *c-erbB-2*; p53; breast cancer; estrogen receptor

INTRODUCTION

Breast carcinoma patients with *c-erbB-2* amplification and/or p53 mutations in the primary tumor have a poorer prognosis [1–3]. Postrecurrence survival is also strongly influenced by amplification of the gene in the primary tumor [4]. Meanwhile, *c-erbB-2* amplification and p53 mutations are strongly related to expression levels of the proteins, which can be determined by immunohistochemical staining or enzyme immunoassay [5–7]. However, it is still unclear how often expression levels of *c-erbB-2* and p53 proteins change as the disease progresses and/or in response to postoperative adjuvant therapies. Re-

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cently, herceptin, a humanized anti-ErbB-2 monoclonal antibody, was reported to be effective against breast carcinomas that showed overexpression of the *c-erbB-2* gene [8]. The p53 tumor suppressor gene was also reported to be a potential target of gene therapy for human cancers [9].

The purpose of this study was to investigate whether expression levels of *c-erbB-2* and p53 proteins in breast cancer tissues differ in primary tumors and their respective metastatic lesions.

MATERIALS AND METHODS

Expression levels of *c-erbB-2* and p53 proteins were examined in 42 breast cancer samples from 21 patients. All patients had undergone radical surgery in our hospital for primary tumors and surgical resection of asynchronous metastatic lesions. These were a consecutive series of patients who had undergone surgical resection of the metastatic tumors between April 1996 and September 1998. The methods of immunohistochemical staining and sandwich enzyme immunoassay have been reported previously [5–7,10]. The immunohistochemical analyses were strictly evaluated according to these methods.

Commercially available polyclonal antibodies against *c-erbB-2* protein (Nichirei Co., Tokyo, Japan) were used for immunohistochemistry. Cell membranes were graded according to the intensity of staining based on the method documented by Tsuda et al. [5]. When the surface of the cell membrane was distinctly stained for *c-erbB-2*, the staining was categorized as strongly positive. When the surface of the cell membrane and cytoplasm were faintly stained, the staining was categorized as weakly positive. The strongly positive cases were judged to have overexpression.

With regard to p53, an anti-p53 rabbit polyclonal antibody (Rsp53, Nichirei) was used for immunohistochemistry of p53 [7]. *c-erbB-2* protein overexpression and p53 immunoreaction were judged as positive when >10% of cancer cells revealed strongly positive membrane and nuclear staining, respectively [5,7]. The histopathological slides were evaluated blindly by the staff pathologist (H.T.) without knowledge of patient identity.

The monoclonal antibodies that react with the extracellular domains of *c-erbB-2* protein, 6G10, and SV-2-61 γ (Nichirei) were used for the sandwich enzyme immunoassay. The cutoff level of the *c-erbB-2* protein was set at 18 ng/ml protein.

Immunohistochemistry and enzyme immunoassay of the *c-erbB-2* protein have been well correlated with the amplification of the *c-erbB-2* proto-oncogene [10]. Immunohistochemical staining of *c-erbB-2* protein was used for Cases 1–17 and sandwich enzyme immunoassay was used for Cases 18–21. However, the paraffin blocks of Cases 12, 13, and 21 were not available for p53 immunohistochemistry. Estrogen receptors (ER) and progesterone receptors (PgR) in the tumors were measured by enzyme immunoassay [11]. The cutoff value of each assay was 10 fmol/mg of protein for ER and 13 fmol/mg of protein for PgR. All clinical data were obtained from the patients' medical charts. Pathology reports included tumor size, the number of lymph node metastases, and histological grade. The histological grades of invasive carcinomas were defined as documented previously [12]. Statistical differences were calculated using the χ^2 test.

RESULTS

The clinicopathological factors of the 21 patients are summarized in Table I. The average age of the patients at initial surgery was 50 years (range, 35–75). Thirteen patients (62%) were premenopausal. Fourteen patients (68%) received postoperative adjuvant therapies. Median disease-free survival time was 19 months (range, 5–104). The resected metastatic lesions included 1 in the liver, 3 in the lung, and 3 in the supraclavicular lymph nodes. The remaining 14 were local skin lesions. The expression levels of *c-erbB-2* and p53 proteins in each tumor are shown in Table II. There was no difference in the positivity rate of *c-erbB-2* (38%: 8/21) and p53 (39%: 7/18) expression between the primary tumors and the recurrent lesions (Table III). In addition, no discordant case of *c-erbB-2* or p53 expression was observed between the primary tumors and their respective metastatic lesions. All 8 primary tumors that overexpressed the *c-erbB-2* protein were of histological grade 3. Positivity rates for ER and PgR were 50% (10/20) and 60% (12/20) for the primary tumors, but only 25% (5/20) and 30% (6/20) for the recurrent lesions, respectively (Table III; $P = 0.1914$ for ER and $P = 0.1120$ for PgR). There was a nonsignificant trend toward loss of hormone receptors in recurrent breast cancers. Five (50%) of 10 ER-positive tumors changed to ER negative, whereas none of 10 ER-negative tumors changed to ER positive. Six (50%) of 12 PgR-positive tumors changed to PgR negative, whereas none of 8 PgR-negative tumors changed to PgR positive.

DISCUSSION

The *c-erbB-2* oncoprotein is recognized as an independent marker of aggressive tumor behavior in breast cancer patients [1,2]. *c-erbB-2* overexpression occurs in 20%–30% of patients with primary breast cancer [5,6]. Overexpression of the *c-erbB-2* protein can be determined easily by immunohistochemical staining or sandwich enzyme immunoassay. We have already reported that the level of *c-erbB-2* protein was correlated with the histological grade, mitotic index, and nuclear atypia but was inversely related with hormone receptor status [3,6].

However, the frequency of changes in *c-erbB-2* overexpression between the primary tumors and their respective recurrent lesions has been unclear. Overexpression of the *c-erbB-2* protein was reported to be higher in stage

TABLE I. Clinicopathological Characteristics of the Breast Cancer Patients*

Case	Age (years)	Menopausal status	TN	Histology	Histological grade	HLNM	LITC	VITC	Adjuvant therapy
1	48	Pre	T2N0	Ductal	2	0/22	−	−	None
2	43	Pre	T3N0	Ductal	1	0/20	−	−	None
3	55	Post	T2N0	Ductal	3	8/24	−	−	CTX
4	46	Pre	T2N0	Ductal	3	11/28	+	−	CTX +H
5	57	Post	T2N0	Ductal	2	2/14	+	−	CTX +H
6	42	Pre	T1N0	Ductal	2	1/19	+	−	CTX +H
7	48	Pre	T2N0	Ductal	3	1/18	+	−	CTX +H
8	75	Post	T2N0	Ductal	2	0/7	−	−	None
9	61	Post	T2N0	Lobular	3	3/28	+	−	CTX +H
10	44	Pre	T4N2	Ductal	3	16/18	+	−	CTX +H
11	44	Pre	T2N0	Ductal	1	0/22	−	−	None
12	45	Pre	T2N0	Ductal	3	3/16	+	−	H
13	43	Pre	T2N0	Ductal	3	0/31	−	−	None
14	42	Pre	T1N0	Ductal	3	0/13	−	−	None
15	35	Pre	T4N1	Ductal	3	15/23	+	−	CTX
16	43	Pre	T3N1	Ductal	3	1/14	+	−	CTX +H
17	74	Post	T1N1	Ductal	3	2/14	−	−	H
18	52	Post	T2N0	Ductal	3	0/16	−	−	CTX
19	52	Pre	T2N1	Ductal	3	2/8	−	−	CTX
20	50	Post	T3N2	Lobular	3	28/31	+	−	None
21	54	Post	T2N1	Ductal	3	0/17	−	−	H

*HLNM, histological lymph node metastasis; LITC, lymphatic invasion by tumor cells; VITC, vascular invasion by tumor cells; CTX, chemotherapy; H, hormone therapy.

TABLE II. *c-erbB-2* and p53 Expression and Hormone Receptor Status in the Primary Tumor and Its Respective Metastatic Lesion*

Case	Metastatic site	Disease-free interval (mo)	*c-erbB-2*/p53		ER/PgR	
			Primary	Metastatic	Primary	Metastatic
1	Local	16	−/−	−/−	+/+	−/−
2	Local	8	−/−	−/−	+/+	−/+
3	Local	6	+/−	+/−	−/−	−/−
4	SCLN	17	+/+	+/+	−/+	−/−
5	Local	63	−/−	−/−	+/+	−/−
6	Local	40	−/−	−/−	+/+	+/+
7	Local	19	−/+	−/+	−/+	−/+
8	Local	23	−/−	−/−	+/+	−/−
9	Liver	25	−/−	−/−	+/+	+/−
10	Local	17	+/+	+/+	−/−	−/−
11	Local	50	−/−	−/−	+/+	−/+
12	Local	12	+/NT	+/NT	−/−	−/−
13	Lung	38	+/NT	+/NT	+/+	+/+
14	SCLN	19	−/+	−/+	−/−	−/−
15	SCLN	23	+/+	+/+	NT/−	NT/NT
16	Local	25	−/−	−/−	−/−	−/−
17	Local	5	−/+	−/+	−/−	−/−
18	Lung	15	−/+	−/+	−/−	−/−
19	Local	14	+/−	+/−	−/−	−/−
20	Local	104	−/−	−/−	+/+	+/−
21	Lung	31	+/NT	+/NT	+/+	+/+

*SCLN, supraclavicular lymph nodes; NT, not tested.

IV cases and in recurrent lesions than in operable breast cancers [6,10]. Recently, Gebhardt et al. [13] reported that the alternatively spliced *c-erbB-2* variant may be involved in the micrometastasis of breast cancer. However, in the present study, there was no difference in the positivity rate of *c-erbB-2* expression between the primary tumors and the recurrent lesions. In addition, no discordant *c-erbB-2* expression was observed between the primary tumors and their respective metastatic lesions. Although breast cancers with the overexpressed

TABLE III. Positive Rates of *c-erbB-2*, p53, and Hormone Receptors in the Primary Tumor and Its Respective Metastatic Lesion

	Primary (%)	Metastatic (%)	*P*
c-erbB-2	8/21 (38)	8/21 (38)	—
p53	7/18 (39)	7/18 (39)	—
ER	10/20 (50)	5/20 (25)	0.1914
PgR	12/20 (60)	6/20 (30)	0.1120

c-erbB-2 gene are more likely to recur, *c-erbB-2* expression in breast cancer cells was unchanged as the disease progressed. We previously reported that *c-erbB-2* amplification was frequently observed in intraductal carcinomas [3]. In the present study, persistence of negative *c-erbB-2* expression in breast cancers could be observed in asynchronous metastatic lesions.

On the basis of these findings, it is suggested that *c-erbB-2* gene amplification occurs in the early stage of breast cancer development. The *c-erbB-2* gene does not appear to be directly linked with the metastatic process in breast cancer. Kuukasjarvi et al. [14] reported that there was a trend toward loss of hormone receptors in recurrent breast cancers. Our results also confirmed this, but we found that overexpression of the *c-erbB-2* oncogene in breast cancer tissues appeared to be stable as the disease progressed. Postoperative adjuvant therapies had no effect on the expression of the gene. Although our study looked mainly at locoregional recurrences, it is possible that the site of recurrence is not related to the degree of overexpression of the gene. Herceptin is indicated only for metastatic breast cancers, in which the primary tumors showed *c-erbB-2* overexpression.

Davidoff et al. [15] reported that p53 mutations of breast carcinomas are also conserved in the lymph node metastases. In the present study, we also confirmed this in the distant metastatic lesions. In addition, our results also indicated that negative p53 immunoreaction could be maintained in asynchronous metastatic lesions. Although it is possible that the p53 tumor suppressor gene may be involved in cell cycle regulations, its role in cancer metastasis has been investigational.

CONCLUSIONS

Although the frequency of overexpression of *c-erbB-2* protein has been reported to be higher in the recurrent lesions than in the primary tumors, in the present study, *c-erbB-2* expression in breast cancer cells was almost unchanged as the disease progressed and/or in response to adjuvant therapies. Expression levels of p53 protein was also stable during the disease progression, whereas the hormone receptor status became negative in the metastatic lesions.

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